

***What Is Claimed Is:***

1. An expression vector comprising:

(a) a first coding sequence operably linked to a tissue-specific transcriptional regulatory sequence wherein the gene product of said first coding sequence is essential for vector replication, wherein said first coding sequence and said tissue-specific transcriptional regulatory sequence are not derived from the same gene; and

(b) at least one additional coding sequence encoding a heterologous gene product, wherein said additional coding sequence is operably linked to said tissue-specific transcriptional regulatory sequence or is operably linked to a second transcriptional regulatory sequence that is activated by the gene product of said first coding sequence.

2. The vector of claim 1, wherein said first and at least one of said additional coding sequences are transcribed from a single tissue-specific transcriptional regulatory sequence.

3. The vector of claim 1, wherein said first coding sequence and at least one of said additional coding sequences are transcribed from separate tissue-specific transcriptional regulatory sequences.

4. The vector of claim 1, wherein at least one of said additional coding sequences replaces a coding sequence in a gene on the vector, which gene is not essential for vector replication, so that said additional coding sequence is operably linked to and transcribed from the transcriptional regulatory sequence from the gene nonessential for vector replication.

5. The vector of claim 1, wherein said tissue-specific transcriptional regulatory sequence is a promoter or an enhancer.

6. The vector of claim 5, where said promoter is selected from the group consisting of CEA, MUC1/DF3,  $\alpha$ -fetoprotein, erb-B2, surfactant, tyrosinase, PSA, TK, p21, and cyclin.

7. The vector of claim 5, wherein said enhancer is selected from the group consisting of DF3, breast cancer-specific enhancer, viral enhancers, and steroid receptor enhancers.

5 8. The vector of claim 1, wherein said additional coding sequence is selected from the group consisting of thymidine kinase, cytosine deaminase, and purine nucleoside phosphorylase.

9. The vector of claim 1, wherein said vector is a DNA virus.

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10. The vector of claim 9, wherein said DNA virus is selected from the group consisting of adenovirus, herpesvirus, papovavirus, papillomavirus, and hepatitis virus.

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11. The vector of claim 10, wherein said DNA virus is an adenovirus.

12. The vector of claim 10, wherein said first coding sequence is selected from the group consisting of the E1a coding sequence, and the E1b coding sequence.

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13. The vector of claim 11, wherein said second coding sequence replaces a coding sequence nonessential for vector replication, so that said second coding sequence is operably linked to a transcriptional regulatory sequence from said gene nonessential for vector replication.

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14. The vector of claim 13, wherein said coding sequence nonessential for vector replication is selected from the group consisting of E3 coding sequences, E4 coding sequences, E1b coding 19kD coding sequence, and E1b55 kD coding sequence.

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15. A cell containing the vector of claim 1.

16. The cell of claim 15, wherein said vector replicates in said cell by means of said tissue-specific regulatory sequence, and in which cell said additional coding sequences are capable of expression.

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17. The cell of claim 15, wherein said cell is a tumor cell or an abnormally proliferating cell.
- 5 18. The cell of claim 17, wherein said additional coding sequence provides a gene product that provides anti-tumor activity in said cell.
19. The cell of claim 17, wherein said tumor cell is selected from the group consisting of a hepatoma cell, and lung carcinoma cell.
- 10 20. A virion containing the vector of claim 1.
21. A cell containing the virion of claim 20, wherein said cell is a producer cell for said virion.
- 15 22. A cell containing the virion of claim 20, wherein said transcriptional regulatory sequence functions in said cell so that replication of the virion and expression of the additional coding sequence occurs in said cell.
23. A cell containing the virion of claim 20, wherein cell is a tumor cell.
- 20 24. A cell containing the virion of claim 23, wherein said heterologous gene product provides anti-tumor activity in said cell.
- 25 25. A method of producing the vector of claim 1, comprising culturing the cell of claim 15 and recovering said vector from said cell.
26. A method of producing the virion of claim 20, comprising culturing the cell of claim 21 and recovering said virion from said cell.
- 30 27. A method for distributing a polynucleotide in a tissue *in vivo*, comprising introducing said vector of claim 1 into said tissue and allowing replication of said vector to occur in said tissue.
- 35 28. The vector of claim 1, wherein said additional coding sequence expresses a gene product that can reduce or eliminate vector replication.

29. The vector of claim 28, wherein said gene product is selected from the group consisting of cytosine deaminase, thymidine kinase, and purine nucleoside phosphorylase.

5 30. A method for modulating the replication of the vector of claim 28, comprising introducing a nucleoside analogue that is phosphorylated by said gene product, when said vector is replicating in a cell.

10 31. A method for expressing a gene in a cell, comprising introducing the vector of claim 1 into a cell, and allowing expression of said additional coding sequences in said cell.

32. The method of claim 30, wherein said cell is a tumor cell or an abnormally proliferating cell.

15 33. A method for diagnosing a cell for the ability to replicate the vector of claim 1 and express said heterologous gene product therefrom, comprising introducing said vector into said cell and assaying said cell for vector replication and gene expression.

20 34. A method for diagnosing a tumor for the ability to replicate the vector of claim 1 and express said heterologous gene product therefrom, and subsequently treating said tumor in a patient, comprising  
25 (a) explanting a tumor biopsy from said patient,  
(b) introducing into the cells of said biopsy the vector of claim 1,  
(c) assaying said vector replication and expression in said cells, and  
(d) introducing said vector into said patient.

30 35. The method of claim 34, further comprising  
(e) adding a nucleoside analogue.

36. The method of claim 35, wherein said nucleoside analogue has anti-tumor activity or eliminates cell proliferation.

35 37. The method of claim 35, wherein said nucleoside analogue is selected from the group consisting of ganciclovir, acyclovir, 1,2-deoxy-2-fluoro- $\beta$ -D-arabinofuranosil-5-iodouracil, and fencytovir.

38. A virion comprising a tissue-specific replication-conditional adenoviral vector comprising:

(a) a heterologous tissue-specific transcriptional regulatory sequence operably linked to the coding region of a gene that is essential for replication of said vector, wherein said coding region is an E1a, E1b, E2, or E4 coding region; and

(b) at least one additional coding sequence encoding a heterologous gene product, wherein said additional coding sequence is operably linked to said heterologous tissue-specific transcriptional regulatory sequence.

39. The virion of claim 38, wherein said tissue-specific transcriptional regulatory sequence is a promoter or an enhancer.

40. The virion of claim 39, where said promoter is selected from the group consisting of an MUC1/DF3 promoter, an alpha-fetoprotein promoter, an erb-B2 promoter, a surfactant promoter, a thymidine kinase promoter, a p21 promoter, and a cyclin promoter.

41. The virion of claim 39, wherein said enhancer is selected from the group consisting of DF3, a breast cancer-specific enhancer, viral enhancers, and steroid receptor enhancers.

42. The virion of claim 38, wherein said additional coding sequence is selected from the group consisting of a thymidine kinase coding sequence, a cytosine deaminase coding sequence, and a purine nucleoside phosphorylase coding sequence.

43. An isolated cell comprising the virion of claim 38.

44. An isolated cell comprising the virion of claim 38, wherein said transcriptional regulatory sequence functions in said cell so that replication

of said virion and expression of said additional coding sequence occurs in said cell.

5           45. The cell of claim 43, wherein said cell is a tumor cell or an abnormally proliferating cell.

46. The cell of claim 45, wherein said additional coding sequence provides a gene product that provides anti-tumor activity in said cell.

10          47. The cell of claim 45, wherein said tumor cell is selected from the group consisting of a hepatoma cell, and lung carcinoma cell.

48. A method of producing the virion of claim 38, comprising culturing a cell infected with said virion and recovering said virion from said cell.

15          49. The virion of claim 38, wherein said additional coding sequence expresses a gene product that can reduce or eliminate virion replication.

20          50. The virion of claim 49, wherein said gene product is selected from the group consisting of cytosine deaminase, thymidine kinase, and purine nucleoside phosphorylase.

51. A virion comprising a tissue-specific replication-conditional adenoviral vector comprising:

25           (a) a heterologous tissue-specific transcriptional regulatory sequence operably linked to the coding region of the adenovirus E1a gene that is essential for replication of said vector; and  
             (b) at least one additional coding sequence encoding a heterologous gene product, wherein said additional coding sequence is operably  
30           linked to a second transcriptional regulatory sequence that is activated by the E1a gene product.

52. The virion of claim 51, wherein said at least one additional coding

sequence replaces a coding sequence of a gene in said vector, which gene is not essential for vector replication, such that said at least one additional coding sequence is operably linked to and transcribed from said second transcriptional regulatory sequence.

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53. The virion of claim 51, wherein at least one of said transcriptional regulatory sequences is a promoter or an enhancer.

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54. The virion of claim 53, where said promoter is selected from the group consisting of an MUC1/DF3 promoter, an alpha-fetoprotein promoter, an erb-B2 promoter, a surfactant promoter, a thymidine kinase promoter, a p21 promoter, and a cyclin promoter.

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55. The virion of claim 53, wherein said enhancer is selected from the group consisting of DF3, a breast cancer-specific enhancer, a viral enhancer, and a steroid receptor enhancer.

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56. The virion of claim 51, wherein said additional coding sequence is selected from the group consisting of a thymidine kinase coding sequence, a cytosine deaminase coding sequence, and a purine nucleoside phosphorylase coding sequence.

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57. The virion of claim 51, wherein said at least one additional coding sequence encodes a gene product that can reduce or eliminate replication of said vector.

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58. The virion of claim 57, wherein said gene product is selected from the group consisting of cytosine deaminase, thymidine kinase, and purine nucleoside phosphorylase.

59. An isolated cell comprising the virion of claim 51.

60. An isolated cell comprising the virion of claim 51, wherein said

transcriptional regulatory sequence operably linked to the coding region of the adenovirus E1a gene functions in said cell so that replication of said virion and expression of said additional coding sequence occurs in said cell.

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61. The cell of claim 59, wherein said cell is a tumor cell or an abnormally proliferating cell.

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62. The cell of claim 61, wherein said at least one additional coding sequence encodes a gene product that provides anti-tumor activity in said cell.

63. The cell of claim 61, wherein said tumor cell is selected from the group consisting of a hepatoma cell and lung carcinoma cell.

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64. A method of producing the virion of claim 51, comprising culturing a cell infected with said vector and recovering said vector from said cell.

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65. The virion of claim 38, wherein said transcriptional regulatory sequence is a tumor-specific regulatory sequence.

66. The virion of claim 65, wherein said tumor-specific regulatory sequence is a tumor-specific promoter.

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67. The virion of claim 38, wherein said transcriptional regulatory sequence is an alpha-fetoprotein promoter.

68. The virion of claim 38, wherein said coding region is the E1a coding region.

69. The virion of claim 38, wherein said coding region is the E1b coding region.

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70. The virion of claim 38, wherein said coding region is an E2 coding region.

71. The virion of claim 70, wherein said coding region is the E2a coding region.



72. The virion of claim 38, wherein said coding region is the E4 coding region.

5 73. The virion of claim 38, wherein said additional coding sequence is a thymidine kinase coding sequence.

74. The cell of claim 43, wherein said transcriptional regulatory sequence is a tumor-specific regulatory sequence.

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75. The cell of claim 74, wherein said tumor-specific regulatory sequence is a tumor-specific promoter.

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76. The cell of claim 43, wherein said transcriptional regulatory sequence is an alpha-fetoprotein promoter.

77. The cell of claim 43, wherein said coding region is the E1a coding region.

78. The cell of claim 43, wherein said coding region is the E1b coding region.

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79. The cell of claim 43, wherein said coding region is an E2 coding region.

80. The cell of claim 79, wherein said coding region is the E2a coding region.

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81. The cell of claim 43, wherein said coding region is the E4 coding region.

82. The cell of claim 43, wherein said additional coding sequence is a thymidine kinase coding sequence.

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83. The virion of claim 51, wherein said transcriptional regulatory sequence operably linked to the coding region of the adenovirus E1a gene is a tumor-specific regulatory sequence.

84. The virion of claim 83, wherein said tumor-specific regulatory sequence operably linked to the coding region of the adenovirus E1a gene is a tumor-specific promoter.

5 85. The virion of claim 51, wherein said transcriptional regulatory sequence operably linked to the coding region of the adenovirus E1a gene is an alpha-fetoprotein promoter.

10 86. The virion of claim 51, wherein said at least one additional coding sequence replaces a coding sequence of the adenovirus E3 gene in said vector, such that said at least one additional coding sequence is operably linked to and transcribed from said second transcriptional regulatory sequence.

15 87. The virion of claim 86, wherein said second transcriptional regulatory sequence is an adenovirus E3 promoter.

20 88. The virion of claim 51, wherein said additional coding sequence is a thymidine kinase coding sequence.

89. The virion of claim 87, wherein said additional coding sequence is a thymidine kinase coding sequence.

25 90. The cell of claim 59, wherein said transcriptional regulatory sequence operably linked to the coding region of the adenovirus E1a gene is a tumor-specific regulatory sequence.

30 91. The cell of claim 90, wherein said tumor-specific regulatory sequence operably linked to the coding region of the adenovirus E1a gene is a tumor-specific promoter.

92. The cell of claim 59, wherein said transcriptional regulatory sequence operably linked to the coding region of the adenovirus E1a gene is an

alpha-fetoprotein promoter.

93. The cell of claim 59, wherein said at least one additional coding sequence replaces a coding sequence of the adenovirus E3 gene in said vector, such that said at least one additional coding sequence is operably linked to and transcribed from said second transcriptional regulatory sequence.

94. The cell of claim 93, wherein said second transcriptional regulatory sequence is an adenovirus E3 promoter.

95. The cell of claim 59, wherein said additional coding sequence is a thymidine kinase coding sequence.

96. The cell of claim 94, wherein said additional coding sequence is a thymidine kinase coding sequence.